# A Review of Low-Intensity Pulsed Ultrasound for Therapeutic Applications

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***Abstract*—Ultrasound therapy has a long history of novel applications in medicine. Compared to high-intensity ultrasound used for tissue heating, low-intensity ultrasound has drawn increasing attention recently due to its ability to induce therapeutic changes without biologically significant temperature increase. Low-intensity pulsed ultrasound (LIPUS) is a specific type of ultrasound that delivers at a low intensity and outputs in the mode of pulsed waves. It has minimal thermal effects while maintaining the transmission of acoustic energy to the target tissue, which is able to provide noninvasive physical stimulation for therapeutic applications. LIPUS has been demonstrated to accelerate the healing of fresh fracture, nonunion and delayed union in both animal and clinical studies. The effectiveness of LIPUS for the applications of soft-tissue regeneration and inhibiting inflammatory responses has also been investigated experimentally. Additionally, research has shown that LIPUS is a promising modality for neuromodulation. The purpose of this review is to provide an overview of the recent developments of LIPUS for therapeutic applications, based on the papers that report positive effects, and to present the findings on the understanding of its mechanism. Current available LIPUS devices are also briefly described in this paper.**

***Index Terms*—Low-intensity pulsed ultrasound, bone healing, soft-tissue regeneration, inflammation inhibition, neuromodulation.**

## I. Introduction

THE use of ultrasound for medical applications including diagnostics, surgery, and therapy, has been investigated for decades [1]. As early as the late 1920s, ultrasound therapy began to be explored by Wood and Loomis [2]. While early applications focused on the thermal effects of ultrasound to selectively raise the temperature of particular tissues, recently, increasing attention has been drawn to its non-thermal effects, which lead to a variety of therapeutic applications.

Manuscript received March 6, 2018; revised July 6, 2018 and November 6, 2018; accepted December 14, 2018. Date of publication December 25, 2018; date of current version September 18, 2019. This work was supported by the Natural Sciences and Engineering Research Council of Canada. *(Corresponding author: Jie Chen.)*

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Digital Object Identifier 10.1109/TBME.2018.2889669

One important parameter of ultrasound is the intensity. Compared to the application of diagnostic imaging, which applies ultrasound at intensities ranging from 0.05–0.5 W/cm$^2$ [3], [4], and the application of ultrasound in surgery with high intensities of 0.2–100 W/cm$^2$ and up to 10,000 $\mathrm{W/cm^2}$ when applying high-intensity focused ultrasound [4]–[6], ultrasound therapy applies both high and low intensities [7]–[9]. The use of therapeutic ultrasound with high intensities primarily leverages its thermal effect [10], [11], while the effectiveness of low-intensity therapeutic treatments is predominated by non-thermal effects, including acoustic cavitation and biological signaling etc. [12].

Low-intensity pulsed ultrasound (LIPUS) is a specific type of ultrasound that delivers at a low intensity and outputs in the mode of pulsed waves. It has been demonstrated to be a non-invasive physical stimulus for therapeutic applications [13]. LIPUS has minimal thermal effects due to its low intensity and pulsed output mode while maintaining the transmission of acoustic energy to the target tissue [14].

As ultrasound is known as a form of acoustic energy at frequencies above the limit of human audibility [15], characterized by frequencies greater than 20 kHz and up to several gigahertz [16], LIPUS generally applies the frequencies of 1-3 MHz, as shown in Fig. 1(a) [17]. Other parameters of LIPUS therapies include the ultrasound intensity ranging from 0.02–1 $\mathrm{W/cm^2}$ spatial average temporal average (SATA) and a treatment duration of 5–20 minutes per day [18]–[22]. Current available LIPUS devices typically deliver ultrasound at an intensity of 30 $\mathrm{mW/cm^2}$ SATA with a frequency of 1.5 MHz, pulsed at 1 kHz with a duty cycle of 20%. A schematic of such LIPUS waveforms is depicted in Fig. 1(b). As shown in Fig. 1(b), the ultrasound has a pulse repetition of 1 ms and is pulsed in a 1:4 mode. The 200 $\mu$s output is followed by an 800 $\mu$s off period. During each 200 $\mu$s pulsing time, the pulse frequency is 1.5 MHz.

LIPUS has a variety of therapeutic applications. It has been demonstrated to stimulate the healing of fresh fracture, nonunion and delayed union both in animal models and in clinical treatments [8], [23], [24]. Its applications for the accelerated healing of fresh fracture and the treatment of established nonunion are approved by the U.S. Food and Drug Administration (FDA) in 1994 and 2000 respectively [25]. LIPUS has also shown effectiveness in soft-tissue regeneration, such as tendon, ligaments and cartilage etc. [19], [20], [26], [27]. Additionally, other applications such as inhibiting inflammatory responses and neuromodulation have been investigated experimentally [28], [29].

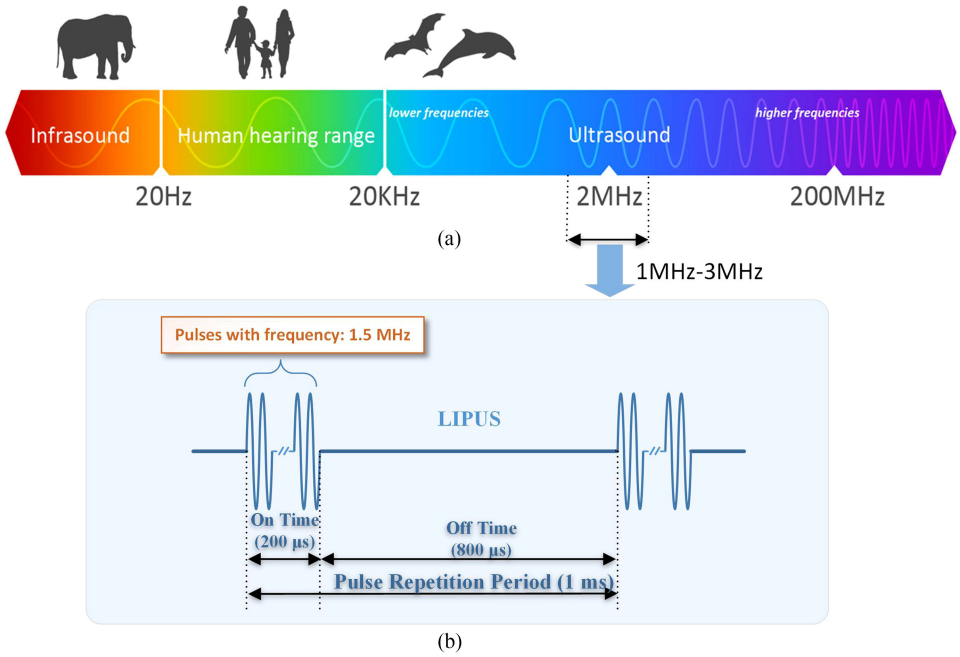



Fig. 1. The range of ultrasonic wave frequencies [17] is shown in (a), of which LIPUS generally applies the frequencies of 1–3 MHz. A schematic example of LIPUS waveforms is shown in (b). It features a pulse frequency of 1.5 MHz and a repetition frequency of 1 KHz with the duty cycle of 20%.

This review offers a systemic overview of evidence-based studies on recent developments of LIPUS in therapeutic applications. While other reviews on ultrasound are available, they usually offer either excessively broad coverage combining both high- and low-intensity waves in all kinds of fields (such as biotechnology, medicine, and diagnostics), or focus on a very narrow therapeutic application (e.g., bone therapy), this review focuses particularly on the therapeutic applications of LIPUS. We compare the status of the LIPUS therapy in each field and discuss the main problems faced by each therapy. In this review, the up-to-date underlying mechanisms are also briefly described to provide insights into the effectiveness of LIPUS. It is followed by a detailed description of therapeutic applications along with the main limitations of existing studies. Current available LIPUS devices are also briefly outlined in this paper.

## II. Therapeutic Mechanisms of LIPUS

### A. Biophysics of LIPUS

LIPUS, a form of mechanical energy at lower intensities, can be transmitted through a medium to cells and tissues as high-frequency acoustic waves [30]. It is generally generated by a transducer converting electrical power to mechanical energy through the mechanism of piezoelectricity. The periodic mechanical sound waves of LIPUS can cause vibrations and collisions by transmission through the medium [4]. It results in minimal thermal and major non-thermal effects in the target [31]. Overall, the non-thermal effects mostly include microbubbles and microjets induced by cavitation, acoustic streaming and mechanical stimulation etc. [12], [32].

### B. Biological Effects of LIPUS

Starting from 1994 when the first LIPUS device got approved by the U.S. FDA for bone healing, a lot of papers started to appear reporting and exploring other potential applications of LIPUS. While many papers focused on the positive results and effectiveness of the LIPUS stimulation, the real mechanisms standing behind the LIPUS effects were much less explored and reported. One of the reasons is that the reported studies varied significantly in protocols and parameters. While the studies with different types of cells showed systemic effects of LIPUS, the difference in cell functions and regulation mechanisms made it difficult to conclude clearly for the pathways affected. Recently, with the increasing attention in LIPUS therapy, more substantial efforts are made to clarify the therapeutic mechanisms of LIPUS through its biological effects. Based on the up-to-date available information, the biological effects of LIPUS generally include the regulation of cell proliferation and differentiation, and the opening of membrane channels etc. More detailed review of the studies is given below. A considerable range of experiments was performed on cells and animals. Statistics from these tests provided insights into how LIPUS works through its biological effects, but a more clear picture is still to be obtained.

Many studies showed evidence that LIPUS could regulate cell proliferation and differentiation. Pluripotent mesenchymal cell line C2C12 was targeted by Ikeda *et al.* in their controlled studies [33]. They found that the LIPUS stimulation can promote Runt-related transcription factor 2 (Runx2) protein expression, and activating the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (p38 MAPK). Korstjens *et al.* found a direct connection between

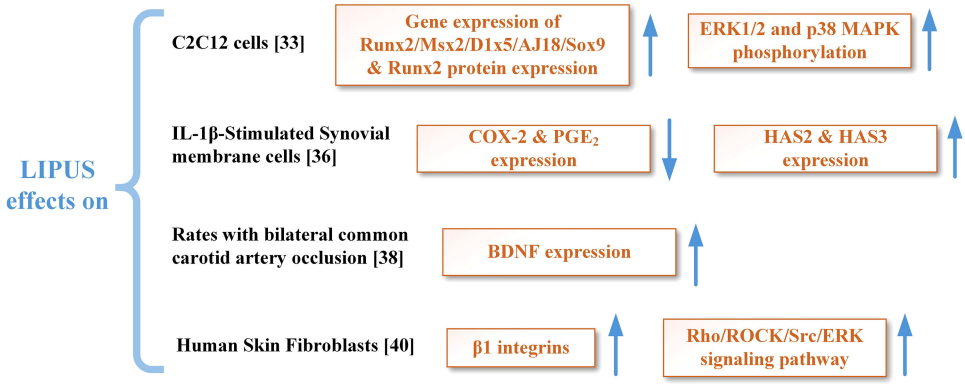



Fig. 2. Several reported biological effects caused by LIPUS on certain targets for different applications.

LIPUS and chondrocyte proliferation and matrix production, and thus LIPUS might provide a feasible tool for cartilage tissue repair in osteoarthritic patients [34]. However, the mechanism is not detailedly presented. Kobayashi *et al.* demonstrated that the cell proliferation and the production of proteoglycan in human nucleus pulposus cell line were stimulated by LIPUS possibly by enhancing growth factor-related genes [35].

For the anti-inflammatory application, Nakamura *et al.* found that LIPUS is able to down-regulate cyclooxygenase-2 (COX-2) and $PGE_2$ expression, and up-regulate HAS2 and HAS3 expression in IL-1$\beta$-stimulated synovial membrane cells [36]. They later reported that LIPUS also suppresses the proliferation and growth of HIG-82 cells stimulated with IL-1$\beta$ or TNF-$\alpha$, and that the LIPUS stimulation may be a medical treatment for joint inflammatory diseases, such as synovitis [37]. With respect to neuromodulation, Huang *et al.* indicated that the therapeutic mechanism of focused LIPUS for neuromodulation is possibly attributed to promoted brain-derived neurotrophic factor (BDNF) expression [38]. The results of these studies can only be regarded as initial immature conclusions. They used uncertain words to describe a possibility. Like the study for neuromodulation in [38], as an animal study, it could only offer some hints to understand the basic mechanisms and cannot substitute for the situation in the human brain. Future research can follow the above studies to investigate the real mechanism.

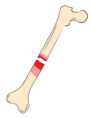
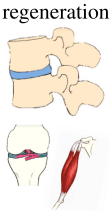
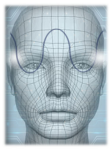
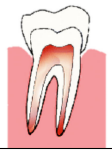
Some researchers investigated changes in transmission channels attributed to the application of LIPUS. Harrison *et al.* determined that the therapeutic mechanism of LIPUS involved integrins which promoted the formation of transmission channels due to focal adhesions in the process of signal conversion [39]. Zhou *et al.* investigated underlying signaling mechanisms of LIPUS in human skin fibroblasts in the role of tissue restoration and remodeling [40]. They found that through the use of LIPUS, the DNA synthesis, and the activation of ERK signaling pathway and $\beta$1 integrins were all significantly upregulated, contributing to cell proliferation. A similar observation of the connection between LIPUS and $\beta$1 integrins was found in human periodontal ligament cells by Hu *et al.* [41]. In terms of signaling, the above studies show a specific picture of the process when LIPUS is applied to the target area. Although it still contains some hypotheses, it helps us understand the mechanism of LIPUS in a dynamic way.

Other researchers focused on combined synergistic bioeffects promoted by LIPUS. Azuma *et al.* [42] found out LIPUS can contribute to the healing of the bone fracture in the states of inflammatory reaction, angiogenesis, chondrogenesis, intramembranous ossification, endochondral ossification, and bone remodeling. Malizos *et al.* concluded that mechanisms of LIPUS for bone healing originates in its biological effect on intracellular activities, cytokine release, the control of related genes, physical effects on bone cell cultures, the acceleration of fluid flow, the generation of micro-stress fields and the facilitation of related enzymatic processes [43]. The potential therapeutic application of LIPUS for urologic diseases was supported by Xin *et al.* such that LIPUS induced multiple bioeffects such as the improvement of related muscle and the nerve regeneration [14]. Through animal and cell experiments, Nakao *et al.* reported that LIPUS not only suppressed the formation of TLR4-MyD88 but also controlled its signal transduction to inhibit inflammation caused by Lipopolysaccharide (LPS) [44]. Similarly, these studies only lie in the area that exists a relationship between LIPUS and its bioeffects for a certain disease. They are too general to understand the details involved in mechanisms. From the results, we could only conclude that LIPUS is related to some specific bioeffects which can help treat some diseases.

The above studies all support the idea that LIPUS as a form of mechanical energy can be applied to clinical therapy. Special proteins are involved in the signaling pathway for different targets. It also activates related cell proliferation and production directly or indirectly. Several reported biological effects of LIPUS described above are summarized in Fig. 2 to present a rough idea that mechanism studies have been done on different targets for different LIPUS applications.

Even though LIPUS has already been investigated in many therapeutic applications experimentally or clinically, continuing investigation is still needed to fully understand its mechanism for safe and efficacious use. Most of the current progress still stays on proving the relationship between LIPUS and different bioeffects. Although they offer positive support for the use of LIPUS for therapy, these studies are still too superficial to uncover the exact nature of its mechanism. For example, it is still unknown what kind of physical stress is related to the bioeffects in LIPUS therapy. Since most of the experiments are based on animals and cells, it lacks studies in humans. Besides,

TABLE I
CURRENT STATUS OF LIPUS FOR DIFFERENT THERAPEUTIC APPLICATIONS

| Application | Target | LIPUS Parameters | Results | Current Status |
|---|---|---|---|---|
| Bone healing  | Fresh Fracture [8], [45] | Typically at 1.5 MHz; 30 $\mathrm{mW/cm^2}$ SATA; 20 % duty cycle at 1 kHz | Reducing fracture healing time and providing clinical benefits, particularly in the circumstances of delayed healing and nonunion | U.S. FDA approved in 1994 and U.K. NICE supported in 2010 |
| | Delayed union [24] | | | U.K. NICE supported in 2010 |
| | Nonunion [23] | | | U.S. FDA approved in 2000 and U.K. NICE supported in 2010 |
| Soft-tissue regeneration  | Tendon [19] | <3 MHz; <1 $\mathrm{W/cm^2}$ no defined application protocol is universally accepted or used, and appropriate dosing of LIPUS treatments needs to be established and validated for achieving optimal results | Promoting tendon healing, ligament healing, intervertebral discs resorption, and cartilage recovery | Pre-clinical studies both *in vivo* and *in vitro* (animal model for all applications while human model has been conducted for cartilage) |
| | Ligaments [20] | | | |
| | Intervertebral discs [46] | | | |
| | Cartilage [27] | | | |
| Inflammation inhibition [29]  | | Beneficial effects were shown with the same parameters used in the clinical treatment of bone fracture. LIPUS with other parameters were also investigated | Preventing periprosthetic inflammatory loosening, inhibiting lipopolysaccharide induced inflammatory responses of osteoblasts and decreasing pro-inflammatory cytokines etc. | Pre-clinical studies both *in vivo* and *in vitro* (animal model) |
| Neuromodulation [28], [47]  | | Effectiveness was shown with the parameters of 1 MHz; 528 $\mathrm{mW/cm^2}$ SPTA; 5% duty cycle at 1 Hz. LIPUS with other parameters were also investigated | Treating traumatic brain injury, vascular dementia and Alzheimer's disease etc. | Pre-clinical studies both *in vivo* and *in vitro* (animal model) |
| Dental treatment [48]  | | Beneficial effects were shown with the same parameters used in the clinical treatment of bone fracture | Promoting periodontal ligaments regeneration, restoring damaged dental roots and decreasing root resorption etc. | Aveo System, a LIPUS device for accelerating orthodontic treatment, was approved by Health Canada in 2016 |

FDA denotes Food and Drug Administration.
NICE denotes National Institute for Health and Care Excellence.
SATA denotes spatial average temporal average.
SPTA denotes spatial peak temporal average.

the existing human studies follow different protocols and do not give sufficient data for a clear picture on the bioeffects. Based on the widely presented positive effects of the therapy, we can expect LIPUS will be widely used in the treatment of different diseases in the future with the help of clinical trials. However, only by understanding the nature of the therapeutic mechanism can LIPUS be applied in treatments without worrying about its potential risk.

## III. THERAPEUTIC APPLICATIONS OF LIPUS

Since its introduction, the potential of ultrasound for clinical applications has attracted significant attention and has been studied extensively by researchers. While the effect of high-intensity ultrasound is associated with generated heat, therapeutic applications of ultrasound are characterized by low intensities within the range of 0.02–1 $\mathrm{W/cm^2}$ SATA and frequencies of 1–3 MHz, the effects of which are not attributed to heat [31].

Therapeutic applications of LIPUS have been studied both *in vitro* and *in vivo*, and promising results gave a way for further investigations in clinical trials. Results have shown positive effects of LIPUS and its potential for broad applications including the areas of bone healing promotion, acceleration of soft-tissue regeneration, inhibition of inflammatory responses and neuromodulation etc. Table I summarizes the current status of LIPUS for different therapeutic applications. The studies covered in the Table I are all associated with the positive effects of LIPUS. However, it's still lack of answers to the appropriate and optimal

parameters and protocols. This section reviews these current or potential therapeutic applications of LIPUS in detail.

### A. Bone Healing

First studies on how ultrasound could be used for bone healing date back to 1952 [49]. Corradi's group performed a *in-vivo* study showing that low-intensity ultrasound enhanced bone formation in rabbits and then clinical trials in 1953, observing the same effect in patients [50]. Since then, repeatable evidence has shown the efficacy of LIPUS in bone treatment. The differences between the conventional ultrasound therapy used in rehabilitation and the LIPUS therapy are the duration of the treatment and the wave parameters. Conventional ultrasound has been used in physiotherapy for decades for improved joint mobility and pain reduction in traumas [51], but mostly due to the thermal effect [11]. Thus, it was characterized by different parameters compared to LIPUS therapy. LIPUS in the therapy of bone healing is usually applied daily and for longer durations compared to the conventional physiotherapy treatment. The LIPUS wave is typically characterized by the frequency of 1.5 MHz, intensity lower than 1 $\mathrm{W/cm^2}$, and 20% repetition duty cycle [52].

The application of LIPUS in the bone treatment has been proven to be effective on every stage of bone fracture healing: decreased inflammation, promoted vascularization, chondrogenesis and ossification, and finally bone remodeling [42]. LIPUS was efficient due to several effects such as stimulation of mesenchymal stem cell recruitment and migration to the fracture sites [53], [54], enhancement of osteoblasts and mesenchymal stem cell differentiation and maturation [55], [56], promotion of matrix calcification, angiogenesis, and callus formation through stimulation of the expression and delivery of several angiogenesis-related cytokines and growth factors [57]–[59], improvement in tissue perfusion at the tendon-bone junction [60], and promotion of endochondral ossification, probably through mechanical forces that are similar to physical loading [61].

With promising results of LIPUS *in vitro* [62], [63], several animal studies on bone fracture healing confirmed the positive effects of LIPUS *in vivo*. Azuma *et al.* studied the effect of LIPUS on rats with bilateral femur fractures by applying ultrasound to the fracture on the right side, while using the left side fracture of the same animal as a control, investigating the effect of LIPUS duration and timing on the healing process [42]. By studying the mechanical properties and histology, they showed that LIPUS accelerated rat femoral fracture healing regardless of timing and duration, and possibly had additive effects [42]. Naruse *et al.* studied the LIPUS effects in combination with teriparatide drug in elderly rats with proven delayed fracture healing compared to the young ones. They observed the same positive effects of the mechanical stimulus of ultrasound wave on promoting faster fracture healing [64]. A Similar study of the LIPUS effects on delayed femoral fracture healing was done on aged mice and showed the same effect of healing time shortening due to ultrasound stimulation [65]. Another study by Naruse *et al.* confirmed that LIPUS accelerated bone healing on aged mice, however, COX-$2^{-/-}$ gene knockout mice did not have the same positive response to mechanical LIPUS treatment, as those in the absence of COX-$2^{-/-}$ modification, and that an immediate early mediator of mechanical stimulation was crucial [66]. Fung *et al.* studied the effect of different distance from the LIPUS transducer to the fracture site on the effect of healing in rats, when ultrasound was applied transcutaneously, and showed that the LIPUS effects were dependent on the depth of fracture under the tissues, and that it should be considered in clinical trials [67]. Wu *et al.* studied the LIPUS effects on osteoporotic rat model showing the same accelerated healing due to LIPUS, and suggesting that LIPUS could be a tool in the osteoporosis treatment [68]. Mayr *et al.* have confirmed the accelerating healing effect of LIPUS on sheep model [69] and Pilla *et al.* on rabbit model [70].

In 1994, the U.S. FDA approval was received on the first LIPUS device for fresh fracture healing [71], [72], and then in 2000 for LIPUS in nonunion treatment [14], [25]. Heckman *et al.* studied the effect of LIPUS as an adjunct therapy for tibial fracture healing in patients in placebo-controlled randomized double-blinded study [51]. They showed both clinically and radiographically, based on the data from 65 fractures, that the LIPUS treated group had much faster fracture healing time, compared to the placebo group. Nolte *et al.* analyzed the data received from the treatment of patients with metatarsal bone fractures, and also showed that the LIPUS treatment results were comparable with the success of surgical treatment, with improved outcomes over more conservative methods [73]. Farkash *et al.* studied the effects of LIPUS in the treatment of delayed union scaphoid fractures and also suggested that, based on their positive results, the LIPUS therapy could serve as an alternative to surgical treatment [24]. LIPUS was shown to increase the healing rate of fresh fractures in the elderly patients, decreasing the chance for nonunion formation [74].

Though nonunions are formed only in 5–10% of the fractures, they fail to heal spontaneously, and the surgical intervention is usually required with the potential risk of complications. Ultrasound stimulation can serve as a non-invasive treatment method providing an alternative to surgery. LIPUS has been shown to increase the healing rate in the treatment of chronic (older than 1 year) nonunions, and it even had positive effects on nonunions that did not heal for up to 10 years [75], [76]. In the treatment of complicated cases of nonunions, it could be used as an adjuvant therapy tool for improving the results of the healing process [77].

Another type of fracture that takes a longer time to heal-osteoporotic bone fractures-pose a serious problem for aging people. The application of ultrasound in their treatment could also enhance the healing process through the same effects: callus formation and remodeling and angiogenesis [78]. Additionally, it also has the potential to be used as a tool to prevent osteoporotic fractures [79].

Today, the LIPUS application in bone fracture healing remains the most studied area as well as the only LIPUS therapy approved by FDA. However, despite the obvious benefits of LIPUS in the healing process, the reported studies varied in conduct and can only be reviewed based on positive effects. Quantification of the effects as well as qualitative analysis of the studies cannot be done with such heterogeneity in

parameters and protocols. Besides, some recent papers started to appear showing null effects from the LIPUS stimulation in their studies and questioning its efficiency [80], [81], [82]. At present, there is only few such papers and there are no studies that tried to compare positive-effect and null-effect studies. Further exploration will be needed in order to make a clear conclusion on the degree of the LIPUS effects alone as well as in combination with other treatments.

The main concern with the reported studies is the lack of unification in parameters, inconsistency in the results and protocols, and heterogenicity of studies (*in vitro*/animal/human). While the LIPUS effects on the healing process are overall beneficial such that it can speed up the healing process and prevent complications, different traumas and conditions may vary significantly in the results after the treatment. Thus it becomes possible to doubt the efficiency of the therapy, and more human randomized clinical trials would be needed to get stronger evidence of the success for the LIPUS therapy in bone healing. Another reason is that the unified mechanism of the LIPUS action still remains unknown. Potential understanding of the mechanism can be a turning point in the selection of optimal parameters for the reliable positive results of the therapy.

### B. Soft-Tissue Regeneration

The positive LIPUS treatment effects on fibroblasts, myoblasts, epithelial cells, and chondrocytes lead to the research for the LIPUS applications in soft-tissue regeneration, including tendon healing, ligament healing, inter-vertebral disc resorption, and cartilage recovery etc.

***1) Tendon:*** As early as 1990, Enwemeka *et al.* studied the potential effects of low-intensity ultrasound in healing tendons [83]. They concluded that compared to high-intensity sonication, ultrasound at low intensities may enhance the healing process of surgically repaired human tendo calcaneus. Histologically, many studies demonstrated the effectiveness of LIPUS for tendon healing, including a better bone mineral density performance and improved tissue integration [84], a remarkable increase in vascularity and a cellular reactive connective tissue [85], and a significant decrease in inflammation and a more regular recovery of scar formation [86]. Vascular endothelial growth factor (VEGF) expression was more complex during the healing stage. Overall, the more mature the healing tissues were, the less VEGF expression was detected in the junction [84]. As to the biomechanical results, failure load, ultimate strength and Vickers hardness of the new bone in the LIPUS group showed a considerable improvement [87]–[89].

LIPUS has been reported to have beneficial effects on bone-tendon healing by promoting fibroblast synthesis [90], collagen formation [91], angiogenic, chondrogenic and osteogenic activities [92]. In 2007, Walsh *et al.* studied the effects of LIPUS in helping heal the tendon-bone interface in an intra-articular sheep knee model [92]. They performed an intra-articular reconstruction and put a single digital extensor tendon autograft on 89 adult wethers. After the LIPUS treatment, all wethers were sacrificed to examine the histology result and mechanical performance. Compared to the control group, the LIPUS group showed a statistically significant improvement at a late stage of the treatment with regards to histology (vascularity, the presence of Sharpey's fibers, and a continuum between tendon and bone) and mechanical performance (stiffness and peak load) [92]. More recently, people also investigated LIPUS for industrial applications. Lovric *et al.* examined the beneficial effects for LIPUS on initial tendon-bone healing using eight transosseous-equivalent mature wethers. Experimental results showed that a continuum grew between the tendon and bone and VEGF, Runx2, and Smad4 all displayed an increase [93].

Multitherapeutic effects of LIPUS on tendon healing have received great interest since the 1990s. Gum *et al.* first proposed combining LIPUS with low-intensity gallium arsenide laser photo stimulation and electrical stimulation [94]. Subsequent literature proposed combing LIPUS with only one treatment approach (LIPUS with functional electrical stimulation or with low-intensity laser therapy) [95]–[97]. Although the combination of the laser and ultrasound treatment enhanced the synthesis of collagen [94], no statistically notable difference was found between the combination group and the single therapy group in improving the biomedical characteristics [94], [95], [97]. On the contrary, the combined therapy group with LIPUS and functional electrical stimulation (FES) showed a notable improvement compared to either LIPUS group or FES group alone, observed from a better remodeling of new formed bone and fibrocartilage zone (larger area, higher bone mineral content and density) [96]. On the subject of biomechanisms, the failure load and the ultimate strength in the LIPUS and FES group are far better than the other three groups [96]. However, the synergistic effects of LIPUS with other treatments still need further investigation. More data and further comparison are required to evaluate the multitehrapeutic efficiency.

Overall, this area remains less studied than the bone healing area, though the positive effects observed are similar as well as conclusions, regarding the potential mechanisms of action. Just like in the bone healing, more randomized human clinical trials with controlled unified parameters are needed to receive more systematic data in support of the therapy both alone and in combination with other methods of stimulation.

***2) Ligaments:*** In 2002, Takakura *et al.* investigated the effect of LIPUS in healing the injured medial collateral ligaments [98]. The experiment was carried out using 13 Sprague Dawley rats with the transection of the bilateral medial collateral ligaments. Ultimate load, stiffness, and energy absorption were all enhanced on the 12th treatment day but not at the 21st treatment day, for which authors presumed that the LIPUS exposure was effective for the early stage of the healing process [98]. Sparrow *et al.* illustrated that the LIPUS therapy might accelerate the early recovery as well as decrease the risk of reinjury [99], with their experiment of LIPUS treated rabbits' ligament transection showing slight improvement by 3 weeks and some structural enhancement by 6 weeks. Warden *et al.* also examined the LIPUS effect by a similar kind of experiment with the same LIPUS signal. They made a conclusion that LIPUS can accelerate but not improve ligament healing [100]. Walsh *et al.* predicted that LIPUS had a promising application prospect on anterior cruciate ligament reconstruction based on the great general improvement at the tendon-bone interface [92], so did Ying *et al.* who demonstrated the same phenomenon in 2012 [60].

Human periodontal ligament cells play a fundamental role in periodontal regeneration. Low intensity ultrasound was observed to have consequential effects on activating connective tissue cells *in vitro*, e.g., human osteoblast-like cell line and human periodontal ligament cells (HPDLCs) [101]. Hu *et al.* also examined that physical stimulation at an appropriate intensity (i.e., LIPUS) promoted osteogenic differentiation of HPDLCs [20]. This process might be associated with the activation of Runx2 and integrin $\beta 1$ with the involvement of p38 MAPK [20], [41].

***3) Inter-vertebral Discs:*** In 2005, Miyamoto *et al.* isolated the nucleus pulposus and annulus fibrosus from bovine coccygeal tissue to examine the effect of LIPUS [102]. Iwashina *et al.* operated a similar experiment. However, they used rabbits instead of bovine intervertebral disc cells [46]. Both research groups agreed that the LIPUS signals increased the proteoglycan (PG) synthesis and PG content. Nevertheless, Iwashina *et al.* found that LIPUS also helped upregulate DNA synthesis and content, which was a negative result in Miyamoto *et al.*'s experiment. Iwabuchi *et al.*'s experiments suggested that LIPUS enhanced the herniated disc resorption through TNF-$\alpha$ and MCP-1 pathways [103]. Omi *et al.* observed that TIMP-1 and MCP-1 in nucleus pulposus cells and macrophages at both protein and gene levels were activated by LIPUS signals [104]. Kobayashi *et al.*'s results demonstrated that the LIPUS stimulation significantly upregulated in the gene expression of both growth factors and their receptors, which indicated that LIPUS was an appropriate method in the area of helping the progression of intervertebral disc degeneration [35]. All this research indicated that LIPUS would be an encouraging supplemental treatment of cell metabolism for intervertebral disc resorption.

***4) Cartilage:*** LIPUS can further rehabilitate the bone by promoting the regeneration of cartilage or endochondral bone by stimulating differentiation and formulation on osteoblasts and ossifying cartilage [61]. A series of research studies evaluated the effect of LIPUS on cartilage healing using different models. Cook *et al.* demonstrated the therapeutic effect of LIPUS on healing osteochondral defects of rabbits *in vivo* in 2001 by varying the daily treatment duration and the total number of treatment days [105]. In their study, increasing the duration of each session led to better histologic quality of cartilage while on the other hand, longer treatment days resulted in less degenerative changes compared to shorter treatment days [105]. Again in 2008, Cook concluded that LIPUS (1.5 MHz, 30 $\mathrm{mW/cm^2}$) had significantly improved the interface cartilage by improving cell morphologic characteristics and the bonding of the interface with a host cartilage separated in a dog model [106]. Jia *et al.* examined the gross appearance grades, histological grades, and the optical density of toluidine blue of the tissue in the bilateral osteochondral defected rabbit model, of which they obtained a similar conclusion [107]. In Naito *et al.*'s study, the LIPUS group showed a significant increase in type II collagen synthesis and the Messenger RNA (mRNA) expression of type II collagen, which suggested a potential mechanism of how LIPUS induces cartilage healing [108].

The above subsections covered the studies of the LIPUS therapy for accelerating soft-tissue regeneration, including tendon healing, ligament healing, inter-vertebral disc resorption, and cartilage recovery. We can see that the application of LIPUS both in soft tissue therapy and soft tissue engineering attracts a lot of attention. This is particularly because the tissues like tendons, ligaments, and cartilage functionally have to sustain significant mechanical load in the body, while LIPUS, applied in the healing process or in the tissue development process, can provide that mechanical force with beneficial effects, aside from the other mechanisms of action. On the other hand, the reviewed studies show a significant diversity of the protocols used: while many research groups try LIPUS applications and report positive effects, it is mostly very inconsistent in parameters and would need systematization. The other concern is that the studies presented are still mostly *in-vitro* or *in-vivo* studies. Unlike hard tissues with FDA's approval for decades, it still needs time and positive results from controlled randomized clinical trials to get reliable evidence in the prospects of the LIPUS therapy on the market.

For now, the studies are more explorative. The quantitative evaluation of the independent studies cannot be done due to the insufficiency of data. Because of the different objects used and the different treatment administration, it would not be possible to compare and quantify the results. Available single studies cannot provide definitive conclusions. It also needs further investigation to understand that through which specific mechanism can LIPUS affect soft tissues. *In-vitro* studies for the ligament tissue engineering are trying to explore which genes can be promoted through LIPUS, but cell studies can only give basic insights into human clinical trials. The real effects of human clinical double-blinded randomized trials will need far better exploration as well as optimization of the protocols and the treatment parameters.

### C. Inhibition of Inflammation

Inflammation is a common response to injury. It plays an important role in the healing process. Nonetheless, when the inflammatory response lasts for too long, it can affect the viability and transcriptional activities of the regeneration process [109]. Researchers found that LIPUS could be a potential modality to counterbalance the cytokines in the inflammation, leading to a more effective treatment.

The therapeutic effect of ultrasound in the treatment of epicondylitis was first demonstrated *in vivo* by Binder *et al.* [110]. They described that the LIPUS treated group showed advantages in aspects of pain score, weight lifting, and grip strength. The inhibiting effect of LIPUS on inflammation was then investigated by Harris's group over the next few years by comparing facial swelling, trismus, pain, and serum C-reactive protein results [111], [112]. They observed for the first time that lower-intensity ultrasound was more beneficial than ultrasound in higher intensities [112]. Moreover, there were many research papers that questioned whether a placebo effect was responsible for the perceived efficacy of LIPUS [113]–[115].

In recent years, an increasing body of research (double-blinded, randomized controlled trials) revealed the mechanism and supported the beneficial response of the LIPUS treatment on inflammation. In 2012 and 2013, Engelmann *et al.* and

Nagata *et al.* studied the effect of pulsed ultrasound on the injured muscle model [116], [117]. It was demonstrated that LIPUS combined with gel dimethylsulfoxide (DMSO) had an inhibitory effect on the related pro-inflammatory cytokines (TNF$\alpha$, IL-1$\beta$, phosphor JNK, and NF$\kappa$B) that were increased due to muscle injury [116]. Victor *et al.* did further research regarding the LIPUS treatment effect on muscle injury [118]. The LIPUS parameters and the muscle injury model they used were the same as in Engelmann's study. Their results showed that LIPUS combined with DMSO-gel and gold nanoparticles significantly decreased pro-inflammatory cytokines. This study also provided evidence that the LIPUS treated group had reduced the possibility of contact with Reactive Oxidative Species, which are known to hasten the inflammatory process. Signori *et al.* also evaluated the same effect on a rat model with a surgical incision in the biceps femoris muscle and obtained positive results [119]. Though the evidence obtained in the studies is valuable and gives insights into the mechanism of action, it still gives a bit of confusion as the LIPUS effect was evaluated as a synergistic effect with other treatments. The hypothesis will need further support and evaluation.

Recent research revealed that osteoblasts play an important role in inflammatory response. Osteoblasts can produce chemokine mRNA, which induces bone tissue inflammation [120]. However, as osteoblasts are mechano-sensitive, it is possible to hypothesize that a mechanical wave signal like LIPUS might be useful in the treatment of inflammatory response. It was stated that inflammatory cells (macrophages and lymphocytes) were sensitized by ultrasound mechanical stress [121], [122]. Their potassium channels [121] and the ability to uptake immunoglobulin G [122] could be regulated by high-frequency mechanical waves. Chemokines, a family of small cytokines act as a chemoattractant to guide the movement of cells (e.g., leukocytes), which play an essential role in the inflammatory process. In 2007, Bandow *et al.* reported that chemokines and the receptor activator of nuclear factor kappa B ligand (RANKL) were expressed by osteoblasts in response to the mechanical stress such as the one induced by LIPUS [123]. The experimental results showed that LIPUS (with 1.5 MHz frequency, 200 $\mu$s burst sine waves at 1 kHz, and 30 $\mathrm{mW/cm^2}$ SATA intensity) significantly improved chemokine mRNA (MCP-1, MIP-1$\beta$, MIP-2, and RANKL) expression in mature osteoblasts [123]. Later in 2010, Bandow *et al.* revealed the molecular mechanisms responsible for the inhibitory effect of LPS (i.e., lipopolysaccharide) on osteoblast differentiation [120] via suppressing the chemokine mRNAs' expression in a Myd88-dependent manner. Then Nakao *et al.* examined how LIPUS inhibited LPS-induced inflammatory response of osteoblasts [44]. The LIPUS parameters were the same as in Bandow's experiment with results showing that after the LIPUS treatment on MC3T3-E1 cells and AD293 cells, mRNA induction by LPS and TLR4-MyD88 complex formation were significantly inhibited. LIPUS also inhibited the inflammatory effects on human periodontal ligament-derived stem cells (HPDLSCs) in an experiment showing that LIPUS significantly blocked the inhibitory effects of porphyromonas gingivalis-derived LPS and IL-1$\beta$ on osteogenesis of HPDLSCs [101], [124].

Nakamura *et al.* and Chung *et al.* examined the potential LIPUS as an effective approach for treating the inflammatory activity of synovitis [37], [125]. It was shown in their studies that LIPUS significantly suppressed the proliferation of cells, the cell growth and the DNA fragmentation (a feature of apoptosis) of synovial membrane HIG-82 cells simulated by cytokine TNF-$\alpha$ and IL-1$\beta$ [37], iNOS and chemoattractant chemokine receptor CCR5 [125]. They also showed that COX-1/2-positive cells, which are absent in healthy tissue and present in the inflamed, were also significantly regulated by LIPUS. Additionally, they showed that the infiltration of inflammatory cells, the synovial hyperplasia, the pannus formation, and the cartilage destruction were significantly reduced by the LIPUS treatment compared to the control group.

For inflammation inhibition with regards to LIPUS, for a long time, doubts existed that it worked mainly as a placebo. However, the studies (also reviewed above) showed that it was not the case and now LIPUS for the inflammation inhibition shows promising prospects. Though less evidence and studies are available in support of the therapy efficiency, compared to the approved LIPUS device for bone repair a long time ago, the studies reviewed here presented positive results from animal studies and clinical trials, focusing on different areas of inflammation as well as on evaluating of LIPUS in combination with other anti-inflammatory drugs for synergistic effects. As the studies mostly focus on the positive effects on the inflamed tissue, the hypothesis that LIPUS can enhance healing and reduce inflammation receives proof. However, the extent of the LIPUS effect on itself or combined with other treatments cannot be evaluated as of now. Shared mechanism of action was observed for anti-inflammation therapy with the soft tissue application (mechanical stress effect on osteoblasts) and with the dental application of LIPUS. Therefore, it also faces the same concerns: with obvious attractiveness of the LIPUS therapy, more significant body of evidence from clinical trials would be needed in the future as well as the clarification of the protocols and parameters that work best for different areas of inflammation, before this modality can get FDA's approval and become the regular tool in patients' treatment.

### *D. Non-Invasive Neuromodulation*

The neuromodulatory effect of ultrasound was detected for the first time by Harvey in 1929 [126], who observed that a high-frequency sonic wave could stimulate turtle and frog heart muscles by exciting neuromuscular activities. Later, studies carried out by Fry [127], [128] suggested that the thermal effect of high-intensity ultrasound could be used to treat movement disorders. His pilot study also predicted that ultrasound had a huge potential in treating Parkinson's disease (PD) and chronic pain. With the advances of ultrasound transducers and focusing methods, researchers in the past decade were able to utilize the non-thermal mechanism of low-intensity ultrasound to stimulate neuron cells and brain circuits [129]. Tyler *et al.* first demonstrated that LIPUS was capable of remotely modulating neuronal circuits by stimulating the action potential and synaptic transmission without elevating the temperature that caused ablation

[129]. Following the initial publication by Tyler *et al.* many research groups have successfully demonstrated the neuroprotective and reversible neuromodulatory effects of the low-intensity ultrasound *in vitro* and *in vivo* [130], [131].

In 1997, the U.S. FDA approved the clinical usage of deep brain stimulation, or DBS, for the treatment of various neurodegenerative-related diseases [132]. Low-intensity ultrasound can serve as a tool for functional neuromodulation, similar to those of conventional pharmacological-, electrical-, magnetic-, and optical-treatments, but without the drawbacks of the latter. For instance, chemical treatments impact the metabolic system and have low or no specificity; the electrical methods like DBS require a surgical procedure for electrode implantation [133]; the transcranial magnetic stimulation provides non-invasive treatments, but suffering from low spatial resolution (1 cm) [134]; the optical neuronal excitation requires genetic alteration [135]. Ultrasound, with task-specific design, can achieve reversible, non-invasive neuromodulation with spatial resolution on the millimeter scale and no thermal effect ($< 0.01$ degree) [131], [136], [137].

One of the latest experiments carried out by Zhao *et al.* has successfully demonstrated that LIPUS could be a powerful tool to prevent neuron degeneration in PD by inhibiting 1-Methyl-4-phenylpyridinium ($MPP^+$) induced neurotoxicity and mitochondria dysfunction [47]. The *in-vitro* LIPUS treatment on PC12 cells prior to $MPP^+$ exposure (a major neurotoxic metabolite of PD [138]) modulated the antioxidative proteins to alleviate the oxidative stress, contributing to the protection of the cells by several pathways including K2P channel and stretched activated ion channel-mediated downstream pathways [47]. The correlation between the ultrasound and the neurotrophic factor has also been investigated in several studies. Wang *et al.* demonstrated that the level of Glial cell line-derived neurotrophic fact (GDNF), which plays a key role in supporting the growths and survival of dopaminergic neurons, had increased in the striatum with the striatal administration of lipid-coated GDNF microsphere in combination with low intensity focused ultrasound stimulation [139]. Their *in-vivo* study on rats suggested that the increase in GDNF level by such method increased both the striatal dopamine and nigral tyrosine hydroxylase level, which improved apomorphine-induced rotations in rats with PD [139]. A similar study carried by Samiotaki *et al.* further proved that low-intensity focused ultrasound promotes neuroregeneration cascades in the nigrostriatal pathway, which subsequently reduced neuron cell death [140].

Despite the lack of trial studies and clinical successes, the articles discussed the possibility of using low-intensity ultrasound to treat and modulate PD, assisting drug dosing, as well as alleviating the symptoms that are related to PD. However, the translation from preclinical studies to clinical trials of LIPUS for PD has significantly less progressed in comparison to ultrasound treatment studies for other neurodegenerative diseases such as Alzheimer's Disease and Major Depression [141], [142].

Neuromodulatory and neuroprotective benefits of LIPUS were demonstrated *in vivo* in several other studies, indicating different potential applications, such as memory loss treatment. The *in-vivo* experiment on permanent bilateral common carotid artery occlusion (BCCAO) treated mice suggested that short-duration (5 minutes and 3 times daily) LIPUS sonication for two weeks significantly increased the BDNF level, while the hippocampus and corpus callosum injuries were alleviated in comparison to the untreated BCCAO mice [38]. The behavioral assessment indicated that LIPUS treatment significantly improved the learning and memory abilities and morphology of mice with vascular dementia [38]. *In-vivo* studies showed that the LIPUS treatment could improve behavioral and histological outcomes of brain-injured mice compared to the control group [28]. Lin *et al.* applied the LIPUS treatment on rats prior to administration of aluminum chloride. In their study, the LIPUS treatment increased the level of BDNF, GDNF, and VEGF, with an attenuated concentration of aluminum in the rat's brain [143]. They also showed that the LIPUS-treated rats exhibited significantly improved memory retention during behavioral tests. Their study results suggested the therapeutic effects of LIPUS could against the aluminum-induced cerebral damage in Alzheimer's disease. Researchers also focused on the therapeutic application of ultrasound to increase the permeability of the brain blood barrier (BBB) for drug delivery. It is possible for the ultrasound to transiently open the BBB that allows for a higher dosage of neuroprotective drugs to be delivered to the target brain region [144], [145]. Other groups further investigated the usage of ultrasound as a therapeutic tool to enhance the effect of gene delivery [146], [147]. Both groups achieved non-viral, non-invasive, ultrasound-mediated gene delivery *in vivo*.

As described above, several groups have been involved in the research of the LIPUS application in neuromodulation. Fig. 3 describes the current progress. The top figure shows the three-step process adopted by most research groups, i.e., the stimulation of the neurons → *in-vivo* tests on mice → clinical trials. As shown in the top figure, transducers are generally placed at the bottom of the vessel to stimulate the neurons for *in-vitro* experiments, and *in-vivo* tests tend to apply the transducer on a mouse's head through gels. Clinical trials are more challenging. Several LIPUS devices have been designed to pursue clinical trials for the neuromodulation application. The bottom figure shows the hand-held LIPUS device designed in our research group (the BINARY Research Group at the University of Alberta). As shown, the LIPUS device is equipped with two transducers. These two transducers are to be placed on the temple area, on which the LIPUS waves stimulate by passing through gels, while the hand-held LIPUS device generates and transmits LIPUS waves. The circuit board of the LIPUS device is also shown in the bottom figure. It consists of a power management circuit, a pulse generator circuit, an amplifier circuit, an impedance matching circuit, and a blue-tooth module for wireless communication.

Variations of LIPUS and other alternatives of low-intensity ultrasound treatments have similar neuromodulation benefits. Hameroff *et al.* used transcranial ultrasound (TUS) on the cranial region of patients with chronic pain [148]. They showed that the mood of the TUS-treated patients was immediately elevated after a 40-minute sonication session and the maximum pain level had been reduced. A joint research group in China applied low-intensity pulsed transcranial ultrasound (pTUS) on rats with distal middle cerebral artery occlusion [149]. It was presented that the pTUS treatment resulted in the reduction of neutrophils in the affected area and the improvement of cerebral blood

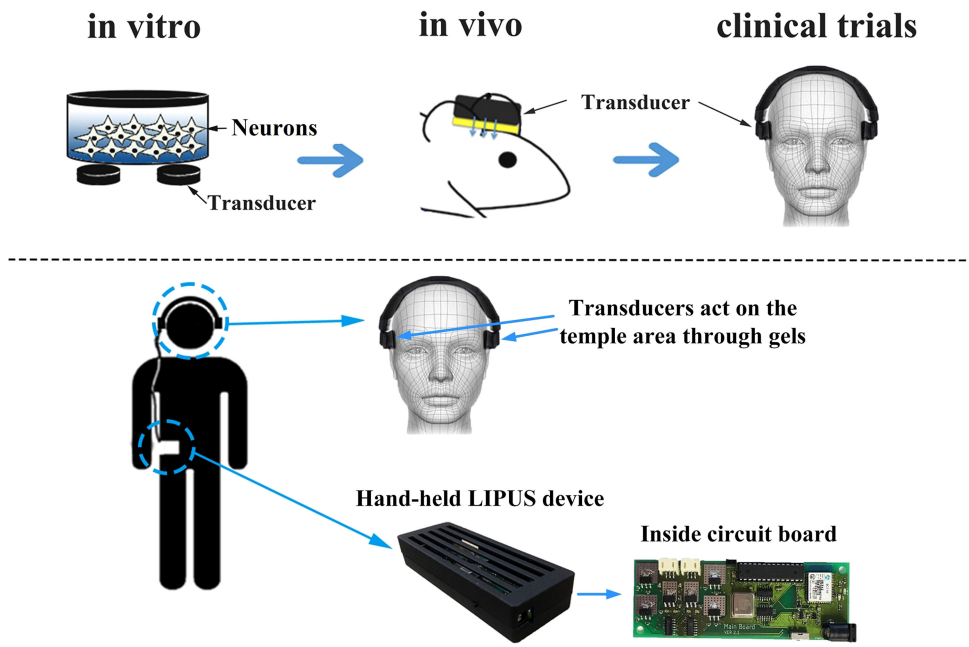



Fig. 3. Current progress of the LIPUS application in neuromodulation. Upper: a common three-step process, i.e., the stimulation of the neurons → *in-vivo* tests on mice → clinical trials. Bottom: a hand-held LIPUS device designed in our research group for clinical trials.

flow that reduced ischemic lesion. The pTUS-treated rats also achieved significantly lower neurological severity score during the behavioral test. This research highlighted the neuroprotective benefits of low-intensity pTUS as an emergency treatment against ischemic brain injury like the stroke. Yoo *et al.* adopted the idea that the ultrasound could suppress the firing between the neurons and the reversibly decrease targeted neuron functionality [150]. Yoo and fellows applied low-intensity pulsed focused ultrasound to the thalamus region of anesthetized rats. The treatment significantly reduced the time of voluntary movements in comparison to the untreated anesthetized rats [150].

Among the reviewed therapeutic areas of the LIPUS application, neuromodulation faces the most challenges. All current studies that were reviewed and emerging patents showed the evidence of the efficiency based on mostly *in-vitro* studies and few animal studies. LIPUS will have to go a long way before reaching to clinical trials. Very few safety studies [13], [151] evaluated possible adverse effects of LIPUS therapy in general and those studies cannot be sufficient to conclude about the LIPUS safety for the stimulation of human brains. New safety evaluations in this area will be required studying both short- and long-term side effects. Despite the challenges (*in-vivo* and clinical trial conductions, safety issues, potential irreversible side effects, etc.) faced by ultrasound in an attempt to find a niche market, LIPUS has shown a potential in neuromodulation and may have a promising future as a cutting-edge technology in high demand.

### *E. Other Applications*

The LIPUS stimulation provides a therapeutic modality for both accelerating bone healing and soft-tissue regeneration, according to Subsections 3.1 and 3.2. This indicates that LIPUS is able to target multiple aspects of tissue engineering, for instance, in the dental field, which involves both hard and soft tissues. This subsection discusses the ability of LIPUS as a multi-target therapy, with the application to dentistry as an example.

Dentistry combines both elements of hard and soft tissues and with the same effects that proved to be helpful for bone healing and soft-tissue regeneration (enhanced angiogenesis, cell proliferation, and differentiation as well as the migration of cells to the wounded site), LIPUS can be applied in the treatment of the teeth and gums [152]. Conventional ultrasound has been used in dentistry back in the 1950s and since then it has been successfully applied for ultrasonic descaling (plaque and other surface deposits removal) [153] and later for imaging as an alternative to X-ray [154]. The LIPUS therapeutic effects attracted research attention much later. Still, studies concerning the effect of LIPUS on dental cells and tissue formation *in vitro* and *in vivo*, as well as studies of LIPUS on human teeth, have been carried out successfully. In particular, many studies were done by the El-Bialy group in the past 10 years, investigating the LIPUS effects in various dental applications (restoring damaged dental roots from stem cells, prevention of further roots degeneration due to gums massage using LIPUS, regeneration of degenerated teeth) both *in vivo* and in patients leading to the filling of a patent and the Health Canada's device approval [155]–[158].

Several potential areas benefited from the therapeutic dental effects of LIPUS were reported. One of them is the application of LIPUS for promoting cementum regeneration, which can be used in the treatment of the root resorption (the loss of root length) [159]. As in the case with osteoblasts and bone healing, mechanical stimulation of cementoblasts (cells forming the tissue covering the root of the teeth) promotes cementoblast differentiation and enhances mineralization of the matrix and

thus prevents root resorption [159]. A study on the effect of LIPUS for the repair of the orthodontically induced tooth resorption in humans was performed in 2004 [157]. It confirmed that LIPUS could promote root healing and hypercementosis, and suggested that LIPUS can be used as a noninvasive tool for root healing.

A study of LIPUS in tooth growth and eruption was performed *in vivo* on rabbits in 2003 [156]. It showed that ultrasound increased the length of tooth segments that consisted of the osteodentin-like tissue and cementum, proving that LIPUS can promote dental tissue growth. The therapeutic potential of LIPUS in dental implantation was also studied *in vivo* and results showed that ultrasound could promote the osseointegration of dental implants and the bone healing around the area of implants [160]–[162]. At the same time, a preliminary *in-vivo* study of the LIPUS effect on dentoalveolar ankylosis was performed by Kiyokawa *et al.* [163]. They showed the potential efficacy of LIPUS in the prevention or the inhibition of ankylosis in re-planted teeth.

Another area is the treatment of the periodontal disease, with LIPUS helping with the regeneration of the root and the periodontal tissue, along with the regeneration of periodontal ligament [164]. Physiologically periodontic ligaments are used for continuous mechanical stress and the ultrasound wave can be used as an additional mechanical force to stimulate cell differentiation and proliferation in tooth regeneration [41], [48].

The soft-tissue repair and the gingival regeneration were also proven to benefit from the LIPUS treatment due to the same effects: the enhancement of connective tissue growth, the promotion of angiogenesis and faster healing [165], [166]. The effect of LIPUS on the soft-dental-tissue healing was evaluated *in vitro* by Iwanabe *et al.* on gingival epithelial cells, and it was shown that LIPUS enhanced cell proliferation and promoted better wound closure [167].

The above description implies that LIPUS provides a promising multi-target therapeutic tool that can be used to enhance both the tooth and the periodontal regeneration in the dental application. Because the dental application of LIPUS combines its efficiency and positive effects explored for both the bone repair and the soft tissue therapy, as well as some effects from the inflammation inhibition, all the same concerns of other therapies are also valid for the dental application. The main problem is the insufficiency of consistent evidence from controlled randomized clinical trials, using the same protocol and parameters. At the same time, as the tooth regeneration studies can rely on some results from the bone repair, this area, though relatively new, progresses fast and attracts a lot of attention, also due to its non-invasive method of action and simple application directly to the area needed. Also, because of the work of the El Bialy group in Canada and other groups who followed them, the research in this area is very systematic. The dental application of LIPUS has gotten the Health Canada's approval, leading it to the second therapeutic area that got the approval for the human application.

Further studies are expected to explore more applications of LIPUS as a therapeutic modality for treating multiple targets in the application of tissue engineering.

## IV. LIPUS Devices

As LIPUS has a much lower intensity than the ultrasound generated from most regular clinical machines, it is possible to make LIPUS devices portable and easy-to-use, facilitating the implementation of home-based rehabilitation interventions. Current available LIPUS devices are mainly designed to accelerate bone healing. The Osteotron IV LIPUS Bone Growth Stimulator manufactured in Japan by ITO CO. LTD, applies LIPUS to accelerate bone healing, including fresh fractures, delayed union, and nonunion [168]. The Osteotron IV device has two transducer outputs, enabling two-channel output capabilities for the treatment of multiple areas. Two more examples of LIPUS devices for accelerating bone healing are the MELMAK LIPUS Device by Melmak GmbH [169] and the Exogen device by Bioventus LLC [170]. In contrast to the Osteotron IV LIPUS bone Growth Stimulator, both the Melmak device and the Exogen device provide only one channel output. Studies based on both the Melmak device [24] and the Exogen device [171] have shown the effectiveness of LIPUS in treating delayed union and nonunion. Daily treatments for 20 minutes are suggested for all devices, with the established dose (1.5 MHz; pulse 200 $\mu$s; delivered at 20% duty cycle at 1 KHz; 30 mW/cm$^2$ SATA).

For the application of enhancing the biology of tooth movement and accelerating orthodontic treatment, the Aevo System [172] is introduced by SmileSonica Inc. This device can enable alveolar bone remodeling and accelerates orthodontic treatment without causing any side effects [173]. The Aevo System is designed with two intra-oral appliances connected to a handheld electronic device. It has three different models for customizable home treatment: Top, Bottom, and Dual. For treatments, patients place the appliances into their mouth for one 20-minute session each day.

A U.S. patent [174] describes a designed LIPUS transmitting device for treating inflammatory disorders. LIPUS is delivered at a non-contact distance to treat inflammation in, including but not limited to, rheumatoid arthritis, juvenile arthritis, bursitis, and spondylitis. Although there is no FDA approved medical devices using pulsed ultrasound in the field of anti-inflammatory therapy, the studies presented in the bone healing treatment under the FDA approval demonstrated that LIPUS can be used in the healing of inflammation through enhancing osteogenesis and chondrogenesis etc. [175].

## V. Conclusion

LIPUS provides a therapeutic tool for various medical applications. Compared to the high-intensity ultrasound used for tissue heating, LIPUS primarily delivers non-thermal effects, including microbubbles and microjets induced by cavitation, acoustic streaming, and mechanical stimulation etc. Except for its biophysical effects, LIPUS has been shown to exhibit beneficial biological effects in a number of peer-reviewed studies. Even though these studies have presented that the underlying mechanisms are mainly associated with the regulation of cell proliferation and differentiation, and the opening of membrane channels, current studies are still not sufficient to understand

how biological effects are produced, and underlying cellular and molecular mechanisms remain to be investigated.

The effectiveness of LIPUS to accelerate the healing of fresh fracture, nonunion and delayed union has been demonstrated by many researchers in both animal and clinical studies. With U.K. NICE supporting the use of LIPUS to reduce fracture healing time in 2010, and U.S. FDA approving LIPUS for fracture healing and the treatment of established nonunion, in 1994 and 2000 respectively, clinical interventions have shown positive experience with LIPUS in treating different types of fractures. The U.S. FDA approved the established dose (1.5 MHz; pulse 200 $\mu$s; delivered at 20% duty cycle; 30 $\mathrm{mW/cm^2}$; 20 minutes daily), of which several LIPUS devices are available in the market.

Research has also shown encouraging results with LIPUS in promoting soft-tissue healing, including tendon, ligaments, inter-vertebral discs, and cartilage etc. Pre-clinical studies both *in vivo* and *in vitro* have demonstrated the effectiveness of LIPUS on different target tissues, by applying various LIPUS doses with the variability of frequency and intensity (typically $< 3$ MHz and $< 1$ $\mathrm{W/cm^2}$). However, no established treatment protocol is universally accepted or used. In order to translate this LIPUS efficacy into human use, appropriate dosing of LIPUS treatments needs to be established and validated.

For dental applications in tissue engineering and inhibition of the inflammatory responses, the LIPUS therapy has shown beneficial effects with the same dose used in the clinical treatment of bone fractures. Similar to the application of promoting soft-tissue healing, these applications still need to present more significant evidence from randomized double-blinded clinical trials in order to go through the process of health administrations' approval.

More recently, LIPUS has been shown as a potential modality for neuromodulation. This finding relies mostly on pre-clinical studies: mostly *in vitro* and few *in vivo*. Safety studies are needed before further proceeding despite that potential designs for clinical trials have been investigated and published in patents.

While most of the reviewed papers show a strong potential of LIPUS and support its positive effects, they lack insights into the successful designs of experiments and suggested optimal parameters, especially that most studies are not backed up by mechanism studies. There are also a few studies that report null effects of LIPUS. It is important to weigh the evidence of the LIPUS effectiveness against the evidence of its deficiency if more studies with null effects are further to appear. The parameter ranges that might induce short- or long-term adverse effects need to be further studied for all therapies.

Overall, while the broad applications and the effectiveness of LIPUS attract a lot of attention to this therapy, a better homogeneity of studies, similar conducts, and generally more data are needed to estimate the average positive effects as well as to give qualitative analysis. Finally, comparisons between the LIPUS therapy alone and its combination with other different treatments are needed, to provide further understanding on whether LIPUS is more efficient as a therapy by itself or as an adjunct therapeutic tool giving enhancement to already existed treatments.

## References


[1] D. Ensminger and L. J. Bond, *Ultrasonics: Fundamentals, Technologies, and Applications*, 3rd ed. Boca Raton, FL, USA: CRC Press, 2011.

[2] R. W. Wood and A. L. Loomis, "The physical and biological effects of high-frequency sound-waves of great intensity," *Philos. Mag.*, vol. 4, pp. 417–436, Sep. 1927.

[3] L. Handolin *et al.*, "The effects of low-intensity pulsed ultrasound on bioabsorbable self-reinforced poly L-lactide screws," *Biomaterials*, vol. 23, no. 13, pp. 2733–2736, Jul. 2002.

[4] A. Khanna *et al.*, "The effects of LIPUS on soft-tissue healing: A review of literature," *Brit. Med. Bull.*, vol. 89, pp. 169–182, Mar. 2009.

[5] N. M. Pounder and A. J. Harrison, "Low intensity pulsed ultrasound for fracture healing: A review of the clinical evidence and the associated biological mechanism of action," *Ultrasonics*, vol. 48, no. 4, pp. 330–338, Aug. 2008.

[6] C. P. Phenix *et al.*, "High intensity focused ultrasound technology, its scope and applications in therapy and drug delivery," *J. Pharm. Pharm. Sci.*, vol. 17, no. 1, pp. 136–153, 2014.

[7] G. ter Haar, "Therapeutic ultrasound," *Eur. J. Ultrasound*, vol. 9, pp. 3–9, Mar. 1999.

[8] S. J. Warden *et al.*, "Can conventional therapeutic ultrasound units be used to accelerate fracture repair?" *Phys. Ther. Rev.*, vol. 4, no. 2, pp. 117–126, Sep. 1999.

[9] G. ter Haar, "Therapeutic applications of ultrasound," *Progr. Biophys. Mol. Biol.*, vol. 93, nos. 1–3, pp. 111–129, Jan.–Apr. 2007.

[10] S. J. Warden, "A new direction for ultrasound therapy in sports medicine," *Sports Med.*, vol. 33, no. 2, pp. 95–107, Feb. 2003.

[11] T. Watson, "Ultrasound in contemporary physiotherapy practice," *Ultrasonics*, vol. 48, no. 4, pp. 321–329, Aug. 2008.

[12] G. Lin *et al.*, "Effects and mechanisms of low-intensity pulsed ultrasound for chronic prostatitis and chronic pelvic pain syndrome," *Int. J. Mol. Sci.*, vol. 17, no. 7, Jul. 2016, Art. no. 1057.

[13] D. L. Miller *et al.*, "Overview of therapeutic ultrasound applications and safety considerations," *J. Ultrasound Med.*, vol. 31, no. 4, pp. 623–634, Apr. 2012.

[14] Z. Xin *et al.*, "Clinical applications of low-intensity pulsed ultrasound and its potential role in urology," *Transl. Androl. Urol.*, vol. 5, no. 2, pp. 255–266, Apr. 2016.

[15] J. Blitz, *Fundamentals of Ultrasonics*, 2nd ed. New York, NY, USA: Plenum Press, 1967.

[16] T. S. Awad *et al.*, "Applications of ultrasound in analysis, processing and quality control of food: A review," *Food Res. Int.*, vol. 48, no. 2, pp. 410–427, Oct. 2012.

[17] O. Savchenko, "Application of low intensity pulsed ultrasound for microbial cell stimulation in bioprocesses and development of carbon-based silver covered filters for microbial cells removal in water systems," Ph.D. dissertation, Dept. Biomed. Eng., Univ. Alberta, Edmonton, AB, Canada, 2017.

[18] A. J. Fontes-Pereira *et al.*, "The effect of low-intensity therapeutic ultrasound in induced fracture of rat tibiae," *Acta. Ortop. Bras.*, vol. 21, no. 1, pp. 18–22, Jan./Feb. 2013.

[19] S. L. J. Júnior *et al.*, "Low-intensity pulsed ultrasound accelerates healing in rat calcaneus tendon injuries," *J. Orthop. Sports Phys. Ther.*, vol. 41, no. 7, pp. 526–531, Jul. 2011.

[20] L. Ren *et al.*, "Involvement of p38 MAPK pathway in low intensity pulsed ultrasound induced osteogenic differentiation of human periodontal ligament cells," *Ultrasonics*, vol. 53, no. 3, pp. 686–690, Mar. 2013.

[21] E. Tanaka *et al.*, "Low-intensity pulsed ultrasound in dentofacial tissue engineering," *Ann. Biomed. Eng.*, vol. 43, no. 4, pp. 871–886, Apr. 2015.

[22] Y. Zhao *et al.*, "Applications of low-intensity pulsed ultrasound to increase monoclonal antibody production in CHO cells using shake flasks or wavebags," *Ultrasonics*, vol. 54, no. 6, pp. 1439–1447, Aug. 2014.

[23] B. G. Dijkman *et al.*, "Low-intensity pulsed ultrasound: Nonunions." *Indian J. Orthop.*, vol. 43, no. 2, pp. 141–148, Apr.–Jun. 2009.

[24] U. Farkash *et al.*, "Low-intensity pulsed ultrasound for treating delayed union scaphoid fractures: case series," *J. Orthop. Surg. Res.*, vol. 10, May 2015, Art. no. 72.

[25] C. Rubin *et al.*, "The use of low-intensity ultrasound to accelerate the healing of fractures," *J. Bone Joint Surg.*, vol. 83-A, no. 2, pp. 259–270, Feb. 2001.

[26] Y.-S. Chan *et al.*, "Using low-intensity pulsed ultrasound to improve muscle healing after laceration injury: An in vitro and in vivo study," *Ultrasound Med. Biol.*, vol. 36, no. 5, pp. 743–751, May 2010.

[27] A. Loyola-Sánchez *et al.*, "Effect of low-intensity pulsed ultrasound on the cartilage repair in people with mild to moderate knee osteoarthritis: A double-blinded, randomized, placebo-controlled pilot study," *Arch. Phys. Med. Rehabil.*, vol. 93, no. 1, pp. 35–42, Jan. 2012.

[28] W.-S. Su *et al.*, "Low-intensity pulsed ultrasound improves behavioral and histological outcomes after experimental traumatic brain injury," *Sci. Rep.*, vol. 7, Nov. 2017, Art. no. 15524.

[29] X. Zhao *et al.*, "Low-intensity pulsed ultrasound (LIPUS) prevents periprosthetic inflammatory loosening through FBXL2-TRAF6 ubiquitination pathway," *Sci. Rep.*, vol. 7, Apr. 2017, Art. no. 45779.

[30] M. H. Repacholi *et al.*, *Ultrasound Medical Applications Biological Effects and Hazard Potential*, 1st ed. New York, NY, USA: Plenum Press, 1987.

[31] K. G. Baker *et al.*, "A review of therapeutic ultrasound: Biophysical effects." *Phys. Ther.*, vol. 81, no. 7, pp. 1351–1358, Jul. 2001.

[32] T. Watson and S. R. Young, "Therapeutic ultrasound," in *Electrotherapy Evidence-Based Practice*. Edinburgh, U.K.: Churchill Livingstone, 2008, pp. 179–200.

[33] K. Ikeda *et al.*, "Effects of low-intensity pulsed ultrasound on the differentiation of C2C12 cells," *Life Sci.*, vol. 79, no. 20, pp. 1936–1943, Oct. 2006.

[34] C. M. Korstjens *et al.*, "Low-intensity pulsed ultrasound affects human articular chondrocytes in vitro," *Med. Biol. Eng. Comput.*, vol. 46, no. 12, pp. 1263–1270, Dec. 2008.

[35] Y. Kobayashi *et al.*, "Low-intensity pulsed ultrasound stimulates cell proliferation, proteoglycan synthesis and expression of growth factor-related genes in human nucleus pulposus cell line," *Eur. Cells Mater.*, vol. 17, no. 3, pp. 15–22, Jun. 2009.

[36] T. Nakamura *et al.*, "Effects of low-intensity pulsed ultrasound on the expression and activity of hyaluronan synthase and hyaluronidase in IL-1 $\beta$-stimulated synovial cells," *Ann. Biomed. Eng.*, vol. 38, no. 11, pp. 3363–3370, Nov. 2010.

[37] T. Nakamura *et al.*, "Low-intensity pulsed ultrasound reduces the inflammatory activity of synovitis," *Ann. Biomed. Eng.*, vol. 39, no. 12, pp. 2964–2971, Dec. 2011.

[38] S. Huang *et al.*, "Protective effect of low-intensity pulsed ultrasound on memory impairment and brain damage in a rat model of vascular dementia," *Radiology*, vol. 282, no. 1, pp. 113–122, Jan. 2017.

[39] A. Harrison *et al.*, "Mode & mechanism of low intensity pulsed ultrasound (LIPUS) in fracture repair," *Ultrasonics*, vol. 70, pp. 45–52, Aug. 2016.

[40] S. Zhou *et al.*, "Molecular mechanisms of low intensity pulsed ultrasound in human skin fibroblasts," *J. Biol. Chem.*, vol. 279, no. 52, pp. 54463–54469, Dec. 2004.

[41] B. Hu *et al.*, "Low-intensity pulsed ultrasound stimulation facilitates osteogenic differentiation of human periodontal ligament cells," *PLoS ONE*, vol. 9, no. 4, Apr. 2014, Art. no. e95168.

[42] Y. Azuma *et al.*, "Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions in the fracture callus," *J. Bone Miner. Res.*, vol. 16, no. 4, pp. 671–680, Apr. 2001.

[43] K. N. Malizos *et al.*, "Low-intensity pulsed ultrasound for bone healing: An overview," *Injury*, vol. 37, no. 1, pp. S56–S62, Apr. 2006.

[44] J. Nakao *et al.*, "Low-intensity pulsed ultrasound (LIPUS) inhibits LPS-induced inflammatory responses of osteoblasts through TLR4–MyD88 dissociation," *Bone*, vol. 58, pp. 17–25, Jan. 2014.

[45] R. W. Poolman *et al.*, "Low intensity pulsed ultrasound (LIPUS) for bone healing: A clinical practice guideline," *BMJ*, vol. 356, Feb. 2017, Art. no. j576.

[46] T. Iwashina *et al.*, "Low-intensity pulsed ultrasound stimulates cell proliferation and proteoglycan production in rabbit intervertebral disc cells cultured in alginate," *Biomaterials*, vol. 27, no. 3, pp. 354–361, Jan. 2006.

[47] L. Zhao *et al.*, "Neuroprotective effect of low-intensity pulsed ultrasound against MPP+-induced neurotoxicity in PC12 cells: Involvement of K2P channels and stretch-activated ion channels," *Ultrasound Med. Biol.*, vol. 43, no. 9, pp. 1986–1999, Sep. 2017.

[48] E. B. Rego *et al.*, "Current status of low intensity pulsed ultrasound for dental purposes," *Open Dent. J.*, vol. 6, pp. 220–225, Dec. 2012.

[49] C. Corradi and A. Cozzolino, "The action of ultrasound on the evolution of an experimental fracture in rabbits," *Minerva Orthop.*, vol. 55, no. 2, pp. 44–45, 1952.

[50] C. Corradi and A. Cozzolino, "Ultrasound and bone callus formation during function," *Arch. Orthop.*, vol. 66, pp. 77–98, 1953.

[51] J. D. Heckman *et al.*, "Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound," *J. Bone Joint Surg. Am.*, vol. 76, no. 1, pp. 26–34, Jan. 1994.

[52] V. Frenkel, *Therapeutic Ultrasound : Mechanisms to Applications*, 1st ed. Hauppauge, NY, USA: Nova Science Publishers, 2010.

[53] F.-Y. Wei *et al.*, "Low intensity pulsed ultrasound enhanced mesenchymal stem cell recruitment through stromal derived factor-1 signaling in fracture healing," *PLoS ONE*, vol. 9, no. 9, Sep. 2014, Art. no. e106722.

[54] F. Y. Wei *et al.*, "Low-magnitude high-frequency vibration enhanced mesenchymal stem cell recruitment in osteoporotic fracture healing through the SDF-1/CXCR4 pathway," *Eur. Cells Mater.*, vol. 31, pp. 341–354, May 2016.

[55] Z.-J. Zhang *et al.*, "The influence of pulsed low-intensity ultrasound on matrix production of chondrocytes at different stages of differentiation: An explant study," *Ultrasound Med. Biol.*, vol. 28, no. 11, pp. 1547–1553, Nov./Dec. 2002.

[56] K. Matsumoto *et al.*, "Low-intensity pulsed ultrasound stimulation promotes osteoblast differentiation through hedgehog signaling," *J. Cell. Biochem.*, vol. 119, no. 6, pp. 4352–4360, Jun. 2018.

[57] L. Li *et al.*, "Low-intensity pulsed ultrasound regulates proliferation and differentiation of osteoblasts through osteocytes," *Biochem. Biophys. Res. Commun.*, vol. 418, no. 2, pp. 296–300, Feb. 2012.

[58] P. Reher *et al.*, "Effect of ultrasound on the production of IL-8, basic FGF and VEGF," *Cytokine*, vol. 11, no. 6, pp. 416–423, Jun. 1999.

[59] A. Suzuki *et al.*, "Daily low-intensity pulsed ultrasound-mediated osteogenic differentiation in rat osteoblasts," *Acta Biochim. Biophys. Sin.*, vol. 41, no. 2, pp. 108–115, Feb. 2009.

[60] Z.-m. Ying *et al.*, "Low-intensity pulsed ultrasound therapy: A potential strategy to stimulate tendon-bone junction healing," *J. Zhejiang Univ. Sci. B*, vol. 13, no. 12, pp. 955–963, Dec. 2012.

[61] P. A. Nolte *et al.*, "Low-intensity ultrasound stimulates endochondral ossification in vitro," *J. Orthop Res.*, vol. 19, no. 2, pp. 301–307, Mar. 2001.

[62] C. M. Korstjens *et al.*, "Stimulation of bone cell differentiation by low-intensity ultrasound - A histomorphometric in vitro study," *J. Orthop Res.*, vol. 22, no. 3, pp. 495–500, May 2004.

[63] B. Trepczik *et al.*, "Endochondral ossification in vitro is influenced by mechanical bending," *Bone*, vol. 40, no. 3, pp. 597–603, Mar. 2007.

[64] K. Naruse *et al.*, "The low-intensity pulsed ultrasound (LIPUS) mechanism and the effect of teriparatide on fracture healing," *J. Orthop. Trauma*, vol. 30, no. 8, pp. S3–S3, Aug. 2016.

[65] M. Katano *et al.*, "Low intensity pulsed ultrasound accelerates delayed healing process by reducing the time required for the completion of endochondral ossification in the aged mouse femur fracture model," *Exp. Anim.*, vol. 60, no. 4, pp. 385–395, 2011.

[66] K. Naruse *et al.*, "Prolonged endochondral bone healing in senescence is shortened by low-intensity pulsed ultrasound in a manner dependent on COX-2," *Ultrasound Med. Biol.*, vol. 36, no. 7, pp. 1098–1108, Jul. 2010.

[67] C.-H. Fung *et al.*, "Investigation of rat bone fracture healing using pulsed 1.5 MHz, 30 $\mathrm{mW/cm^2}$ burst ultrasound - Axial distance dependency," *Ultrasonics*, vol. 54, no. 3, pp. 850–859, Mar. 2014.

[68] S. Wu *et al.*, "Low-intensity pulsed ultrasound accelerates osteoblast differentiation and promotes bone formation in an osteoporosis rat mode," *Pathobiology*, vol. 76, no. 3, pp. 99–107, May 2009.

[69] E. Mayr *et al.*, "Radiographic results of callus distraction aided by pulsed low-intensity ultrasound," *J. Orthop. Trauma*, vol. 15, no. 6, pp. 407–14, Aug. 2001.

[70] A. A. Pilla *et al.*, "Non-invasive low-intensity pulsed ultrasound accelerates bone healing in the rabbit," *J.Orthop. Trauma*, vol. 4, no. 3, pp. 246–253, 1990.

[71] K.-S. Leung *et al.*, "Complex tibial fracture outcomes following treatment with low-intensity pulsed ultrasound," *Ultrasound Med. Biol.*, vol. 30, no. 3, pp. 389–395, Mar. 2004.

[72] G. J. Della Rocca, "The science of ultrasound therapy for fracture healing," *Indian J. Orthop.*, vol. 43, no. 2, pp. 121–126, Apr.–June 2009.

[73] P. Nolte *et al.*, "Heal rate of metatarsal fractures: A propensity-matching study of patients treated with low-intensity pulsed ultrasound (LIPUS) vs. surgical and other treatments," *Injury*, vol. 47, no. 11, pp. 2584–2590, Nov. 2016.

[74] R. Zura *et al.*, "A cohort study of 4,190 patients treated with low-intensity pulsed ultrasound (LIPUS): Findings in the elderly versus all patients." *BMC Musculoskelet Disord.*, vol. 16, Mar. 2015, Art. no. 45.

[75] D. Gebauer *et al.*, "Low-intensity pulsed ultrasound: Effects on nonunions," *Ultrasound Med. Biol.*, vol. 31, no. 10, pp. 1391–1402, Oct. 2005.

[76] R. Zura *et al.*, "Treatment of chronic ($>$1 year) fracture nonunion: Heal rate in a cohort of 767 patients treated with low-intensity pulsed ultrasound (LIPUS)," *Injury*, vol. 46, no. 10, pp. 2036–2041, Oct. 2015.

[77] Y. Watanabe *et al.*, "Three key factors affecting treatment results of low-intensity pulsed ultrasound for delayed unions and nonunions: instability, gap size, and atrophic nonunion," *J. Orthop. Sci.*, vol. 18, no. 5, pp. 803–810, Sep. 2013.
[78] W.-H. Cheung *et al.*, "Low-intensity pulsed ultrasound accelerated callus formation, angiogenesis and callus remodeling in osteoporotic fracture healing," *Ultrasound Med. Biol.*, vol. 37, no. 2, pp. 231–238, Feb. 2011.
[79] D. Lim *et al.*, "Low-intensity ultrasound stimulation prevents osteoporotic bone loss in young adult ovariectomized mice," *J. Orthop. Res.*, vol. 29, no. 1, pp. 116–125, Jan. 2011.
[80] P. Martinez de Albornoz *et al.*, "The evidence of low-intensity pulsed ultrasound for in vitro, animal and human fracture healing," *Brit. Med. Bull.*, vol. 100, no. 1, pp. 39–57, Dec. 2011.
[81] J. W. Busse *et al.*, "Re-evaluation of low intensity pulsed ultrasound in treatment of tibial fractures (TRUST): randomized clinical trial," *Brit. Med. J.*, vol. 355, Oct. 2016, Art. no. i5351.
[82] S.-W. Yang *et al.*, "Does low-intensity pulsed ultrasound treatment repair articular cartilage injury? A rabbit model study," *BMC Musculoskelet Disord.*, vol. 15, Feb. 2014, Art. no. 36.
[83] C. S. Enwemeka *et al.*, "The biomechanical effects of low-intensity ultrasound on healing tendons," *Ultrasound Med. Biol.*, vol. 16, no. 8, pp. 801–807, 1990.
[84] H. Lu *et al.*, "Low-intensity pulsed ultrasound accelerated bone-tendon junction healing through regulation of vascular endothelial growth factor expression and cartilage formation," *Ultrasound Med. Biol.*, vol. 34, no. 8, pp. 1248–1260, Aug. 2008.
[85] L. K. Papatheodorou *et al.*, "Effect of transosseous application of low-intensity ultrasound at the tendon graft-bone interface healing: Gene expression and histological analysis in rabbits," *Ultrasound Med. Biol.*, vol. 35, no. 4, pp. 576–584, Apr. 2009.
[86] B. S. Gan *et al.*, "The effects of ultrasound treatment on flexor tendon healing in the chicken limb," *J. Hand Surg.*, vol. 20, no. 6, pp. 809–814, Dec. 1995.
[87] H. Lu *et al.*, "Low-intensity pulsed ultrasound accelerates bone-tendon junction healing: A partial patellectomy model in rabbits," *Am. J. Sports Med.*, vol. 34, no. 8, pp. 1287–1296, Aug. 2006.
[88] L. Qin *et al.*, "Low intensity pulsed ultrasound increases the matrix hardness of the healing tissues at bone-tendon insertion-a partial patellectomy model in rabbits," *Clin. Biomech. (Bristol, Avon)*, vol. 21, no. 4, pp. 387–394, May 2006.
[89] L. Qin *et al.*, "Low-intensity pulsed ultrasound accelerates osteogenesis at bone-tendon healing junction," *Ultrasound Med. Biol.*, vol. 32, no. 12, pp. 1905–1911, Dec. 2006.
[90] J. Rantanen *et al.*, "Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle injury," *Am. J. Sports Med.*, vol. 27, no. 1, pp. 54–59, Jan./Feb. 1999.
[91] A. Da Cunha *et al.*, "The effect of therapeutic ultrasound on repair of the achilles tendon (tendo calcaneus) of the rat," *Ultrasound Med. Biol.*, vol. 27, no. 12, pp. 1691–1696, Dec. 2001.
[92] W. R. Walsh *et al.*, "Effects of low-intensity pulsed ultrasound on tendon-bone healing in an intra-articular sheep knee model," *Arthroscopy*, vol. 23, no. 2, pp. 197–204, Feb. 2007.
[93] V. Lovric *et al.*, "The effects of low-intensity pulsed ultrasound on tendon-bone healing in a transosseous-equivalent sheep rotator cuff model," *Knee Surg. Sports Traumatol. Arthrosc.*, vol. 21, no. 2, pp. 466–475, Feb. 2013.
[94] S. L. Gum *et al.*, "Combined ultrasound, electrical stimulation, and laser promote collagen synthesis with moderate changes in tendon biomechanics," *Am. J. Phys. Med. Rehabil.*, vol. 76, no. 4, pp. 288–296, Jul./Aug. 1997.
[95] H. Demir *et al.*, "Comparison of the effects of laser, ultrasound, and combined laser + ultrasound treatments in experimental tendon healing," *Lasers Surg. Med.*, vol. 35, no. 1, pp. 84–89, Jul. 2004.
[96] J. Hu *et al.*, "Combined application of low-intensity pulsed ultrasound and functional electrical stimulation accelerates bone-tendon junction healing in a rabbit model," *J. Orthop Res.*, vol. 32, no. 2, pp. 204–209, Feb. 2014.
[97] V. T. Wood *et al.*, "Collagen changes and realignment induced by low-level laser therapy and low-intensity ultrasound in the calcaneal tendon," *Lasers Surg. Med.*, vol. 42, no. 6, pp. 559–565, Aug. 2010.
[98] Y. Takakura *et al.*, "Low-intensity pulsed ultrasound enhances early healing of medial collateral ligament injuries in rats," *J. Ultrasound. Med.*, vol. 21, no. 3, pp. 283–288, Mar. 2002.
[99] K. J. Sparrow *et al.*, "The effects of low-intensity ultrasound on medial collateral ligament healing in the rabbit model," *Am. J. Sports Med.*, vol. 33, no. 7, pp. 1048–1056, Jul. 2005.
[100] S. J. Warden *et al.*, "Low-intensity pulsed ultrasound accelerates and a nonsteroidal anti-inflammatory drug delays knee ligament healing," *Am. J. Sports Med.*, vol. 34, no. 7, pp. 1094–1102, Jul. 2006.
[101] J. Harle *et al.*, "Effects of ultrasound on the growth and function of bone and periodontal ligament cells in vitro," *Ultrasound Med. Biol.*, vol. 27, no. 4, pp. 579–586, Apr. 2001.
[102] K. Miyamoto *et al.*, "Exposure to pulsed low intensity ultrasound stimulates extracellular matrix metabolism of bovine intervertebral disc cells cultured in alginate beads," *Spine*, vol. 30, no. 21, pp. 2398–2405, Nov. 2005.
[103] S. Iwabuchi *et al.*, "In vitro evaluation of low-intensity pulsed ultrasound in herniated disc resorption," *Biomaterials*, vol. 26, no. 34, pp. 7104–7114, Dec. 2005.
[104] H. Omi *et al.*, "Low-intensity pulsed ultrasound stimulation enhances TIMP-1 in nucleus pulposus cells and MCP-1 in macrophages in the rat," *J. Orthop. Res.*, vol. 26, no. 6, pp. 865–871, Jun. 2008.
[105] S. D. Cook *et al.*, "Improved cartilage repair after treatment with low-intensity pulsed ultrasound," *Clin. Orthop. Relat. Res.*, vol. 391, pp. S231–S243, Oct. 2001.
[106] S. D. Cook *et al.*, "The effect of low-intensity pulsed ultrasound on autologous osteochondral plugs in a canine model," *Am. J. Sports Med.*, vol. 36, no. 9, pp. 1733–1741, Sep. 2008.
[107] X.-L. Jia *et al.*, "Effects of low-intensity pulsed ultrasound in repairing injured articular cartilage," *Chin. J. Traumatol.*, vol. 8, no. 3, pp. 175–178, Jun. 2005.
[108] K. Naito *et al.*, "Low-intensity pulsed ultrasound (LIPUS) increases the articular cartilage type II collagen in a rat osteoarthritis model," *J. Orthop. Res.*, vol. 28, no. 3, pp. 361–369, Mar. 2010.
[109] J. G. Tidball and S. A. Villalta, "Regulatory interactions between muscle and the immune system during muscle regeneration," *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, vol. 298, no. 5, pp. R1173–R1187, May 2010.
[110] A. Binder *et al.*, "Is therapeutic ultrasound effective in treating soft tissue lesions?" *Brit. Med. J.*, vol. 290, no. 6467, pp. 512–514, Feb. 1985.
[111] M. Elhag *et al.*, "The anti-inflammatory effects of dexamethasone and therapeutic ultrasound in oral surgery," *Brit. J. Oral Maxillofac. Surg.*, vol. 23, no. 1, pp. 17–23, Feb. 1985.
[112] I. Hashish *et al.*, "Anti-inflammatory effects of ultrasound therapy: Evidence for a major placebo effect," *Rheumatology*, vol. 25, no. 1, pp. 77–81, Feb. 1986.
[113] A. P. D'Vaz *et al.*, "Pulsed low-intensity ultrasound therapy for chronic lateral epicondylitis: a randomized controlled trial," *Rheumatology*, vol. 45, no. 5, pp. 566–570, May 2005.
[114] H. J. Flax, "Ultrasound treatment of peritendinitis calcarea of the shoulder," *Am. J. Phys. Med.*, vol. 43, no. 3, pp. 117–124, 1964.
[115] E. E. Mueller *et al.*, "A placebo-controlled study of ultrasound treatment for periarthritis," *Am. J. Phys. Med.*, vol. 33, no. 1, pp. 31–35, 1954.
[116] J. Engelmann *et al.*, "Pulsed ultrasound and dimethylsulfoxide gel treatment reduces the expression of pro-inflammatory molecules in an animal model of muscle injury," *Ultrasound Med. Biol.*, vol. 38, no. 8, pp. 1470–1475, Aug. 2012.
[117] K. Nagata *et al.*, "Ultrasound modulates the inflammatory response and promotes muscle regeneration in injured muscles," *Ann. Biomed. Eng.*, vol. 41, no. 6, pp. 1095–1105, Jun. 2013.
[118] E. G. Victor *et al.*, "Pulsed ultrasound associated with gold nanoparticle gel reduces oxidative stress parameters and expression of pro-inflammatory molecules in an animal model of muscle injury," *J. Nanobiotechnol.*, vol. 10, Mar. 2012, Art. no. 11.
[119] L. U. Signori *et al.*, "Haematological effect of pulsed ultrasound in acute muscular inflammation in rats," *Physiotherapy*, vol. 97, no. 2, pp. 163–169, Jun. 2011.
[120] K. Bandow *et al.*, "Molecular mechanisms of the inhibitory effect of lipopolysaccharide (LPS) on osteoblast differentiation," *Biochem. Biophys. Res. Commun.*, vol. 402, no. 4, pp. 755–761, Nov. 2010.
[121] D. K. Martin *et al.*, "Human macrophages contain a stretch-sensitive potassium channel that is activated by adherence and cytokines," *J. Membr. Biol.*, vol. 147, no. 3, pp. 305–315, Oct. 1995.
[122] J. Mattana *et al.*, "Repetitive mechanical strain suppresses macrophage uptake of immunoglobulin G complexes and enhances cyclic adenosine monophosphate synthesis," *Am. J. Pathol.*, vol. 147, no. 2, pp. 529–540, Aug. 1995.

[123] K. Bandow *et al.*, "Low-intensity pulsed ultrasound (LIPUS) induces RANKL, MCP-1, and MIP-1$\beta$ expression in osteoblasts through the angiotensin II type 1 receptor," *J. Cell. Physiol.*, vol. 211, no. 2, pp. 392–398, 2007.

[124] J. Kusuyama *et al.*, "Low-intensity pulsed ultrasound (LIPUS) promotes BMP9-induced osteogenesis and suppresses inflammatory responses in human periodontal ligament-derived stem cells," *J. Orthop. Trauma.*, vol. 31, no. 7, pp. S4–S4, Jul. 2017.

[125] J.-I. Chung *et al.*, "Anti-inflammatory effect of low intensity ultrasound (LIUS) on complete Freund's adjuvant-induced arthritis synovium," *Osteoarthritis Cartilage.*, vol. 20, no. 4, pp. 314–322, Apr. 2012.

[126] E. N. Harvey, "The effect of high frequency sound waves on heart muscle and other irritable tissues," *Am. J. Physiol.*, vol. 91, pp. 284–290, Dec. 1929.

[127] W. J. Fry, "Ultrasound in neurology," *Neurology*, vol. 6, no. 10, pp. 693–704, Oct. 1956.

[128] W. J. Fry, "Use of intense ultrasound in neurological research," *Am. J. Phys. Med.*, vol. 37, no. 3, pp. 143–147, Jun. 1958.

[129] W. J. Tyler *et al.*, "Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound," *PLoS ONE*, vol. 3, no. 10, Oct. 2008, Art. no. e3511.

[130] F. J. Fry *et al.*, "Production of reversible changes in the central nervous system by ultrasound," *Science*, vol. 127, no. 3289, pp. 83–84, Jan. 1958.

[131] Y. Tufail *et al.*, "Transcranial pulsed ultrasound stimulates intact brain circuits," *Neuron*, vol. 66, no. 5, pp. 681–694, Jun. 2010.

[132] P. Gildenberg, *Neuromodulation*, 1st ed. San Diego, CA, USA: Academic, 2009.

[133] K. J. Ressler and H. S. Mayberg, "Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic," *Nature Neurosci.*, vol. 10, no. 9, pp. 1116–1124, Sep. 2007.

[134] T. Wagner *et al.*, "Noninvasive human brain stimulation," *Annu. Rev. Biomed. Eng.*, vol. 9, pp. 527–565, Aug. 2007.

[135] S. Szobota *et al.*, "Neurotechnique: Remote control of neuronal activity with a light-gated glutamate receptor," *Neuron*, vol. 54, pp. 535–545, May 2007.

[136] A. Bystritsky *et al.*, "A review of low-intensity focused ultrasound pulsation," *Brain Stimul.*, vol. 4, no. 3, pp. 125–136, Jul. 2011.

[137] M. Fini and W. J. Tyler, "Transcranial focused ultrasound: a new tool for non-invasive neuromodulation," *Int. Rev. Psychiatry*, vol. 29, no. 2, pp. 168–177, Feb. 2017.

[138] W. Dauer and S. Przedborski, "Parkinson's disease: Mechanisms and models," *Neuron*, vol. 39, no. 6, pp. 889–909, Sep. 2003.

[139] X. Wang *et al.*, "Intracerebral administration of ultrasound-induced dissolution of lipid-coated GDNF microbubbles provides neuroprotection in a rat model of Parkinson's disease," *Brain Res. Bull.*, vol. 103, pp. 60–65, Apr. 2014.

[140] G. Samiotaki *et al.*, "Enhanced delivery and bioactivity of the neurturin neurotrophic factor through focused ultrasoundmediated bloodbrain barrier opening in vivo," *J. Cereb. Blood Flow Metab.*, vol. 35, no. 4, pp. 611–622, Mar. 2015.

[141] S. Seidl and J. A. Potashkin, "The promise of neuroprotective agents in Parkinson's disease," *Front Neurol.*, vol. 2, Nov. 2011, Art. no. 68.

[142] L. Kalia *et al.*, "Disease-modifying strategies for Parkinson's disease," *Movement Disorder*, vol. 30, no. 11, pp. 1442–1450, Sep. 2015.

[143] W.-T. Lin *et al.*, "Protective effects of low-intensity pulsed ultrasound on aluminum-induced cerebral damage in Alzheimer's disease rat model," *Sci. Rep.*, vol. 5, Apr. 2015, Art. no. 9671.

[144] S. Raymond *et al.*, "Ultrasound enhanced delivery of molecular imaging and therapeutic agents in Alzheimer's disease mouse models," *PLoS ONE*, vol. 3, no. 5, May 2008, Art. no. e2175.

[145] J. F. Jordão *et al.*, "Amyloid-$\beta$ plaque reduction, endogenous antibody delivery and glial activation by brain-targeted, transcranial focused ultrasound," *Experimental Neurology*, vol. 248, pp. 16–29, Oct. 2013.

[146] C. Lin, H. Hsieh *et al.*, "Focused ultrasound-induced blood-brain barrier opening for non-viral, non-invasive, and targeted gene delivery," *J. Control. Release.*, vol. 212, pp. 1–9, Aug. 2015.

[147] C. Fan *et al.*, "Noninvasive, targeted and non-viral ultrasound-mediated GDNF-plasmid delivery for treatment of Parkinson's disease," *Sci. Rep.*, vol. 6, Jan. 2016, Art. no. 19579.

[148] S. Hamerof *et al.*, "Transcranial ultrasound (TUS) effects on mental states: A pilot study," *Brain Stimul.*, vol. 6, no. 3, pp. 409–415, May 2013.

[149] T. Guo *et al.*, "Pulsed transcranial ultrasound stimulation immediately after the ischemic brain injury is neuroprotective," *IEEE Trans. Biomed. Eng.*, vol. 62, no. 10, pp. 2352–2357, Oct. 2015.

[150] S.-S. Yoo *et al.*, "Transcranial focused ultrasound to the thalamus alters anesthesia time in rats," *Neuroreport*, vol. 22, no. 15, pp. 783–787, Oct. 2011.

[151] F. Ahmadi *et al.*, "Bio-effects and safety of low-intensity, low-frequency ultrasonic exposure," *Prog. Biophys Mol. Biol.*, vol. 108, no. 3, pp. 119–138, Apr. 2012.

[152] K. Lim *et al.*, "In vitro effects of low-intensity pulsed ultrasound stimulation on the osteogenic differentiation of human alveolar bone-derived mesenchymal stem cells for tooth tissue engineering," *BioMed Res. Int.*, vol. 2013, Jul. 2013, Art. no. 269724.

[153] A. D. Walmsley, "Applications of ultrasound in dentistry," *Ultrasound Med. Biol.*, vol. 14, no. l, pp. 7–14, 1988.

[154] J. Marotti *et al.*, "Recent advances of ultrasound imaging in dentistry—A review of the literature," *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.*, vol. 115, no. 6, pp. 819–832, Jun. 2013.

[155] K. S. Alhazmi *et al.*, "Ultrasound enhances dentoalveolar remodeling in an ex vivo orthodontic, ovariectomy-induced osteoporotic model," *Ultrasound Med. Biol.*, vol. 43, no. 9, pp. 1963–1974, Sep. 2017.

[156] T. El-Bialy *et al.*, "Effect of ultrasound on rabbit mandibular incisor formation and eruption after mandibular osteodistraction," *Am. J. Orthod. Dentofac. Orthop.*, vol. 124, no. 4, pp. 427–434, Oct. 2003.

[157] T. El-Bialy *et al.*, "Repair of orthodontically induced root resorption by ultrasound in humans," *Am. J. Orthod. Dentofac. Orthop.*, vol. 126, no. 2, pp. 186–93, Aug. 2004.

[158] T. El-Bialy *et al.*, "Ultrasound effect on neural differentiation of gingival stem/progenitor cells," *Ann. Biomed. Eng.*, vol. 42, no. 7, pp. 1406–1412, Jul. 2014.

[159] D. Pavlin and J. Gluhak-Heinrich, "Effect of mechanical loading on periodontal cells." *Crit. Rev. Oral Biol. Med.*, vol. 12, no. 5, pp. 414–424, 2001.

[160] Y. Ustun *et al.*, "Effects of low-intensity pulsed ultrasound on dental implant osseointegration: A preliminary report." *Eur. J. Dent.*, vol. 2, no. 4, pp. 254–262, Oct. 2008.

[161] S.-K. Hsu *et al.*, "Effects of near-field ultrasound stimulation on new bone formation and osseointegration of dental titanium implants in vitro and in vivo," *Ultrasound Med. Biol.*, vol. 37, no. 3, pp. 403–416, Mar. 2011.

[162] Q. Liu *et al.*, "The effect of low-intensity pulsed ultrasound on the osseointegration of titanium dental implants," *Brit. J. Oral. Maxillofac. Surg.*, vol. 50, no. 3, pp. 244–250, Apr. 2012.

[163] T. Kiyokawa *et al.*, "A preliminary study of effects of low-intensity pulsed ultrasound (LIPUS) irradiation on dentoalveolar ankylosis," *J. Oral Sci.*, vol. 59, no. 3, pp. 447–451, 2017.

[164] T. Inubushi *et al.*, "Effects of ultrasound on the proliferation and differentiation of cementoblast lineage cells," *J. Periodontol.*, vol. 79, no. 10, pp. 1984–1990, Oct. 2008.

[165] H. Ikai *et al.*, "Low-intensity pulsed ultrasound accelerates periodontal wound healing after flap surgery," *J. Periodontal Res.*, vol. 43, no. 2, pp. 212–216, Apr. 2008.

[166] R. Shiraishi *et al.*, "The effects of low-intensity pulsed ultrasound exposure on gingival cells," *J. Periodontol.*, vol. 82, no. 10, pp. 1498–1503, Oct. 2011.

[167] Y. Iwanabe *et al.*, "The effect of low-intensity pulsed ultrasound on wound healing using scratch assay in epithelial cells," *J. Prosthodont Res.*, vol. 60, no. 4, pp. 308–314, Oct. 2016.

[168] Ito Co. Ltd., "Ultrasound Bone Growth Stimulator OSTEOTRON IV," 2018. [Online]. Available: http://www.itocoltd.com/products/osteotron4/

[169] Innovative Medical Supplies, "MELMAK$^{TM}$ Low Intensity Pulsed Ultrasound (LIPUS) Device Bone Stim," 2018. [Online]. Available: https://innovativemedicalsupplies.com/product/melmak-low-intensity-pulsed-ultrasound-lipus-device-bone-stim#.Wh9sp0qnGUl

[170] Bioventus LLC., "exogen: ultrasound bone healing system," 2014. [Online]. Available: http://www.exogen.com/can-e

[171] X. Roussignol *et al.*, "Indications and results for the Exogen$^{TM}$ ultrasound system in the management of non-union: A 59-case pilot study," *Orthop. Traumatol. Surg. Res.*, vol. 98, no. 2, pp. 206–213, Apr. 2012.

[172] SmileSonica Inc., "Aevo System," 2018. [Online]. Available: http://aevosystem.com/

[173] SmileSonica Inc., "Orthodontists | Aevo System," 2018. [Online]. Available: http://aevosystem.com/orthodontists/

[174] M. T. Peterson, "Methods for treating inflammatory disorders," U.S. Patent US20 090 177 123A1, Jul. 9, 2009.

[175] B. Simpson, "Low Intensity Pulsed Ultrasound," *In Touch*, no. 137, pp. 2–11, 2011. [Online]. Available: https://physioclinic.net/wp-content/uploads/2015/04/LIPUS-Article-In-Touch-2011.pdf